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Echocardiographic reference ranges for sedentary Donkeys in the UK

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Manuscript prepared for *Veterinary Record*

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Abstract

The aim of this study was to provide 2D and M-mode echocardiographic reference ranges from a sample of the UK population of donkeys including geriatrics (>30 years), owned by The Donkey Sanctuary, and to assess the influence of gender, weight and age on these variables. A total of 36 donkeys with no clinical or echocardiographic evidence of cardiovascular disease were examined; 24 geldings and 12 females, aged 3 – 45 years old, weighing 130 – 262 kg. Left atrial to aortic ratio was larger in geldings (P=0.004). There was no significant difference for left ventricular M-mode diastolic diameter between females and geldings (P=0.121) after exclusion of one heavy female outlier. 2D measurements significantly increased with body weight including maximal left atrial diameter (R^2= 0.112; P=0.046), aortic diameter at various levels (e.g. annulus: R^2=0.35; P<0.001) and the pulmonary artery diameter (R^2=0.124; P=0.035). M-mode measurements were not significantly influenced by weight other than the left ventricular free-wall in systole (R^2=0.118; P=0.041). Age and heart rate did not have any significant effect on echocardiographic variables. This is the first UK study to report on echocardiographic reference ranges of sedentary donkeys across a wide age range, and shows differences compared to reference ranges from working donkeys.

Key words

Equus asinus, Cardiology, cardiac ultrasound, reference ranges

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Introduction

The total number of donkeys in the UK is estimated to be 10 000. Approximately 35% of these are owned by The Donkey Sanctuary (Starkey and Starkey 1996) some of which are loaned out (“adopted”) by independent carers (Cox and others 2010). Compared with the estimated 44 million donkeys worldwide (Starkey and Starkey 1996), UK donkeys are not likely to be working (other than some leisure donkeys) and their role is as a pet, companion or in animal assisted therapy for people with special needs (Morrow and others 2011). Although the average age of working donkeys has been reported as <15 years (Pritchard and others 2005), UK donkeys have been estimated to live at least 20 years (Cox and others 2010) and at The Donkey Sanctuary, the average age of death in one study was 30.5 years (Burden and others 2008).

Echocardiography is increasingly available as a non-invasive tool to screen for or diagnose cardiac disease in all species. Echocardiographic studies of healthy donkeys have been published in the literature to provide reference intervals, both for non-working (Amory and others 2004; Delvaux and others 2001) and working animals (Hassan and Torad 2015). However numbers of animals and the young age of donkeys included in these studies (n=20; 4 – 18 years (Amory and others 2004); n=12; 2 – 18 years (Delvaux and others 2001); n=30; 2 – 6 years (Hassan and Torad 2015) respectively) mean that echocardiographic data regarding healthy geriatric donkeys and donkeys with cardiac disease are lacking.

It is known in horses and humans that athletic cardiac remodelling can occur, identified by echocardiography (Buhl and others 2005; Giada and others 1998; Young and others 2005), reversible on de-training (Giada and others 1998; Kriz and others 2000), so it is unlikely that echocardiography reference data from working donkeys can be applied to sedentary donkeys. Furthermore, ageing is known to influence echocardiographic measurements in humans, even in the absence of systemic hypertension or acquired cardiac disease, with smaller left ventricular (LV) lumen and thicker LV walls (Gardin and others 1979). Indeed, normograms (Henry’s) are published for echocardiographic reference ranges in humans, taking into account their age (Feigenbaum 1994). The influence of ageing on echocardiographic measurements has not been explored in equidae, other than the age-related development of degenerative valve disease resulting in aortic regurgitation. Furthermore, horses do not live as long as donkeys, so donkeys may be more likely to demonstrate the effect of ageing on their echocardiographic variables.

Therefore, the aim of this study was to provide 2D and M-mode echocardiographic reference ranges in donkeys with a wide age range, and to explore the effects of age, gender, body mass and body condition score on these echocardiographic variables.

Materials and Methods

Donkeys

Donkeys in this observational cross-sectional study were selected from a population of 202 donkeys at the Donkey Sanctuary which had undergone physical examination and auscultation (findings from the whole groups detailed in parallel manuscript: Roberts & Dukes-McEwan). Donkeys were selected from this population by a veterinary surgeon at The Donkey Sanctuary, from a wide range of ages, to give a fairly even distribution within the age groups <15 years, 15 – 30 years and >30 years, located on one farm. A further 6 donkeys were assessed by the author (SLR) at different locations at The Donkey Sanctuary, under similar husbandry conditions. Donkeys were excluded from this echocardiographic reference group if they had any audible heart murmur and any significant valvular regurgitation noted on colour flow Doppler echocardiography (CFDE), affecting any valve (Roberts & Dukes-McEwan). Other exclusion criteria were known cardiac disease or a record of previous detection of a heart murmur, significant systemic disease which may affect the cardiovascular system, skin disease affecting the right hemithorax which would preclude clipping for echocardiography, and those donkeys non-compliant with handling. Chronic laminitis and dental disease were not considered exclusion criteria.

2D and M-mode Echocardiography

Donkeys had their right thoracic limb one stride forward (or held by a groom). Acoustic preparation was achieved with clipping over the cardiac area on the right hemithorax, cleaning with hibitane and wiping with surgical spirit, followed by liberal application of ultrasound gel. The investigator was seated during the examination, on the right side of the donkey. Transthoracic echocardiography was performed using an Esaote MyLab30Vet Gold ultrasound machine with a 2.5-3.5 MHz phased array sector transducer. Standard cardiac 2D right parasternal long and short axis views were obtained in standing donkeys as previously described for horses (Marr and Patteson 2010). An electrocardiogram was recorded simultaneously to time cardiac events, with leads placed in a loose base apex configuration (electrodes were ECG pads, placed cranial to right scapular (red) and over left brisket (yellow) with the earth lead at the right thoracic inlet). The presence of structural heart disease and cardiac remodelling was assessed by 2D echocardiography. CFDE was used to exclude anything other than trivial or mild valvular regurgitation (Marr and Patteson 2010; Marr and Reef 1995; Reef 1995). From short axis views of the left ventricle at chordae tendinae level, M-mode echocardiograms were obtained by placing the M-mode cursor to bisect the left ventricular cavity. Images were archived and subsequently reviewed and measured off-line. M-mode measurements followed standard protocol (Sahn and others 1978), using the leading edge to leading edge method (only including one proximal endocardium in each measurement). End-diastole was defined as the start of the QRS complex and end-systole as the nadir of septal motion. Two dimensional measurements at the heart base short axis, optimised for the aortic valve and left atrium, were obtained. From still frames during any point in diastole, usually early in diastole where the cusps of the closed aortic valve leaflets could be clearly visualised, the aortic diameter and left atrial diameter were measured, using inner edge - inner edge methods, as described for dogs (Hansson and others 2002), using the line between left coronary and non-coronary cusps of the aortic valve to define the line of measurement for both aortic diameter and left atrial diameter (Figure 1A). From a right parasternal long axis 4 chamber view, with the left atrium optimised, the maximum left atrial diameter was measured at end-systole (frame before mitral valve opening) (Figure 1B). From the right parasternal long axis left ventricular outflow (5 chamber) view, aortic diameter was measured in systole at the level of the annulus, sinus of Valsalva and sino-tubular junction (Figure 1C). From a right parasternal cranial short axis view, optimised for the pulmonary trunk, the pulmonary annulus diameter was measured in systole.

For each echo variable, at least three cardiac cycles, consecutive if possible, were measured and the mean values recorded. Cardiac cycles associated with arrhythmias (e.g. following 2^{nd} degree atrioventricular block) were not included.

Statistical methods

Data were recorded in an Excel spreadsheet. Data were imported into SigmaPlot 13.0 for Windows (Systat software Inc.). Basic descriptive data are reported as means and standard deviation if data were normally distributed or as medians (minimum – maximum) if not. The Shapiro-Wilk test was used to assess for normality. Comparisons between males and females used the Student's unpaired two-tailed T-test for normally distributed data, or the Mann-Whitney test for non-normal distribution of data or where there was unequal variance. Although donkeys were selected to be from the three different age groups (<15 years, 15 – 30 years and >30 years), age was used as a continuous variable. Linear regression was used to explore associations between sets of variables, such as age and body weight, on echocardiographic data.

A P value of <0.05 was accepted as representing statistical significance.

Results

A total of 53 donkeys underwent echocardiography. Four of these had heart murmurs and another 10 were identified on CFDE to have aortic insufficiency (AoI) (Roberts & Dukes-McEwan), so were excluded from this echocardiographic reference population. One donkey was withdrawn because of dull demeanour and extensive skin disease. Another two were excluded because of incomplete echocardiographic studies. Three donkeys had chronic laminitis, included in the study, but none had other significant systemic disease. Therefore, a total of 36 donkeys underwent echocardiography, with a minimum of 10 in each age group (<15 years (N=13), 15 – 30 years (N=12) and >30 years (n=11)). All had normal echocardiograms based on 2D, M-mode and CFDE assessment.

The description of the donkeys is shown in Table 1. There were 24 males, all geldings, and 12 females. Donkeys were aged between 3 and 45 years old and weighed between 130 – 262 kg. Heart rate documented during echocardiography was a median of 48.8 beats per minute (bpm) (range 38 – 70). There was no statistically significant difference between geldings and females in age, body weight or heart rate (Table 1). However, one female donkey, the largest in the study at 262 kg, 71 kg heavier than the next heaviest female (191 kg) and 56 kg heavier than the next heaviest male (206 kg), was an outlier. If she was excluded, males (175.92 $\pm$ 21.33 kg) were significantly heavier than the majority of females (157.46 $\pm$ 20.80 kg) (P=0.022). Body condition scores were only available for 30/36 donkeys (Table 1), and was 3.0 in 24 of these donkeys, with 5 donkeys scoring 3.5 and one donkey 4.5. Therefore, the effect of BCS was not explored further on the echocardiographic variables, given the lack of variance recorded.

The M-mode echocardiography results for all the donkeys are shown in Table 2A. The only M-mode variable with a significant difference between genders was the LV diastolic diameter (LVDd), which was greater in females (Table 2B) (Figure 2). However, there was one outlying data point, with a 6 year old female, the largest donkey evaluated at 262 kg, having an 8.6 cm diameter left ventricle. If this donkey was excluded, the female mean (± SD) LVDd was 7.0 cm (± 0.5), not significantly different from the male LVDd (6.7 ± 0.6 cm) (P = 0.121).

The 2D echocardiography results for all donkeys are shown in Table 3A. There was no difference between genders for short axis aortic diameter or long axis aortic diameters at valve, sinus of Valsalva or sino-tubular junction level (Table 3B). However, male donkeys had significantly greater short axis left atrial diameter than females which also similarly affected the short axis left atrial / aortic (LA/Ao) ratio (Table 3B).

The effect of weight on echocardiographic variables was explored (Table 4). For M-mode variables, a statistically significant association between measurements and bodyweight was not evident, other than the systolic left ventricular free-wall thickness (Figure 3A). In contrast, however, all the 2D variables (long axis maximal left atrial diameter (systole), short axis left atrial diameter in diastole (Figure 3B), short axis diastolic aortic diameter, long axis aortic systolic diameter at valve annulus (Figure 3C), sinus of Valsalva and sinotubular junction levels and pulmonary artery annulus diameter (Figure 3D) showed significant weak-moderate positive associations with body weight (Table 4).

Heart rate did not have any significant association with any of the M-mode or 2D variables. However, the 2D ratio of the aortic systolic diameter at valve level and the pulmonary artery diameter at valve level was weakly but significantly positively associated with heart rate (R=0.381; R^2 = 0.145; P=0.026).

Despite a non-significant trend to increasing LV diastolic diameter and free wall thickness with advancing age, age did not show any statistically significant associations with any of the M-mode or 2D echocardiographic variables.

Discussion

This is the first UK study to report on echocardiographic reference ranges from sedentary donkeys with a wide age range, including a number of geriatric donkeys. Echocardiographic variables however, show very similar results to those shown in other studies of sedentary donkeys (Amory and others 2004; Delvaux and others 2001). Furthermore, in this study, we did not demonstrate any significant association with age on any of the echocardiographic variables, in contrast to data from humans. Although not statistically significant, there were trends to increasing LV chamber diameter in diastole and systole with age in this study, which is converse to that reported in humans, who have smaller chambers and thicker walls with advancing age (Feigenbaum 1994; Kou and others 2014). There is little information in the veterinary literature about the effect of ageing on echocardiographic dimensions in any species, although wall thickness increases in Cavalier King Charles spaniels without a significant change in LV chamber diameter (Misbach and others 2014). Despite the wide age range of donkeys in this study, these data suggest that age does not have to be taken into account when comparing echocardiographic values with reference values.

As in horses and other species, body size should be taken into account in assessing echocardiographic data with reference ranges. From our data, however, there were no significant associations with body weight on most of the M-mode variables. This is in contrast to significant linear regression associations shown previously in donkeys (Amory and others 2004), including working donkeys (Hassan and Torad 2015). In the study by Amory and others (2004), there was a wider weight range (98 – 255 kg) than this study, which may explain the discordant finding, but the weight range was slightly more narrow in the Hassan and Torad (2015) study (150 – 220 kg) than ours (130 – 262 kg). Even though the number of donkeys included in our study is higher than the other studies, the linear regression analyses were statistically under-powered, which means a genuine influence of body weight on M-mode variables was simply not detected. The lack of a significant influence of body weight on M-mode echocardiographic data is an advantage in the field, however, as weighing scales may not be available at most premises. We did demonstrate significant positive associations with 2D measured variables and body weight, including the left atrium, aortic diameters at the various levels and the pulmonary artery diameter.

These data show that gender does not need to be taken into account in assessing most echocardiographic measurements, at least between geldings and females, as jacks (entire males) were not included. There was no statistically significant difference in body weights between the two genders in this population. However, there was a heavy, outlying female donkey, which, if excluded, resulted in males being significantly heavier. Indeed, there were no significant gender population differences or for most echocardiographic variables which is why echocardiographic data were combined for both genders. In our study, the left atrium (short axis diastolic) diameter was significantly greater in geldings than females, which also affected the LA/Ao ratio. However, this is not likely to be of clinical importance. In humans, the larger left atrium in males than females is no longer evident after normalising for body surface area (Kou and others 2014).

The echocardiographic data generated in this study, although similar to other data from sedentary donkeys (Amory and others 2004; Delvaux and others 2001), show some marked differences compared with data generated in another study of 30 working donkeys (Hassan and Torad 2015). Those donkeys were of similar weight range to those reported here, but the mean M-mode diameters of the LV in diastole (LVDd) and systole (LVDs) are markedly smaller (LVDd range: 3.84 – 5.83 cm; LVDs range: 2.13 – 3.44 cm) than this study (LVDd: 5.4 – 8.6 cm; LVDs: 2.9 – 5.1 cm). As LV wall thickness in diastole and systole from both studies were similar, the relative wall thickness (LVFWd /LVDd ratio) is higher in the working donkeys, calculated from the mean values (1.42/4.82) as 0.29, than the sedentary donkeys in this study (1.7/6.8), at 0.25. This could represent the effect of work and physiological remodelling. However, methodological differences between the two studies is also possible, The figure showing M-mode acquisition in the study of Hassan and Torad (2015) suggests a more apical level of the LV than chordal level, which may result in the smaller LV cavity. Furthermore, in healthy horses of a range of body sizes, level of work reported by owners did not appear to significantly influence echocardiographic results (Al-Haidar and others 2013), although other studies do demonstrate remodelling changes in horses with training (Buhl and others 2005; Young 1999), but with increases in the LV chamber dimensions, rather than reduction. This may reflect the different work that donkeys do, compared with horses. More information is required on the effect of work (and type of work) on the echocardiographic variables in the donkey. The effect of breed of donkey should also be investigated in future work, not investigated in our study of Standard donkeys. A homogenous breed (Baladi Egyptian donkeys) were assessed by Hassan and Torad (2015).

Allometric scaling from body weight is increasingly advocated in the generation of echocardiographic reference ranges (Cornell and others 2004), and this has been proposed for horses of a wide range of body weights (Al-Haidar and others 2013). The donkeys in this study had measured and calculated measurements for LVDd and LVDs which were not significantly different between actual and calculated measurements, but they did not correlate and there were very wide limits of agreement on Bland-Altman plots (data not shown). Future work to generate data from donkey (possibly donkey breed) specific allometric scaling reference ranges based on weight or body size would be useful to refine echocardiographic reference ranges. Indexing chamber sizes to aortic diameter has also been advocated to normalise for patient size, which shows similar results across species (cats, dogs, horses) (Brown and others 2003). This approach may also applied to donkeys in future work.

There are limitations of this study. Although 36 donkeys were included in the study, some statistical methods were under-powered, so larger numbers would have strengthened the data from this study. Due to time constraints, and reluctance to impose further on The Donkey Sanctuary staff, we did not repeat echocardiograms, so intra- and inter-observer echocardiographic repeatability was not assessed, although this is important to know before inferring changes with serial echocardiography, or in interpreting deviations from normal data in an individual case. Only standard M-mode and 2D measurements were obtained for this study; we did not generate reference ranges for spectral Doppler, which may also be important in investigating the effects of ageing or disease. Finally, as body condition scores were very similar in the population, with only one over-weight and no poor-condition donkeys, the influence of BCS on echocardiographic variables could not be assessed.

Conclusions

Standard M-mode and 2D echocardiographic reference ranges generated from 36 UK donkeys of wide age range are similar to those reported for other sedentary donkeys. Body weight did not significantly affect M-mode variables, but did influence 2D variables, particularly of aortic and pulmonary artery diameters, and this should be considered, particularly with very small or very large donkeys. This study showed that as age, gender and heart rate did not have any important influence on echocardiographic variables, they do not need to be considered when comparing patient data to these reference values.

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305 **TABLES**

306 **Table 1: Population description (N=36)**

	Mean ± SD (or median)	Confidence interval of the mean	Minimum - Maximum	
Gender				24 G; 12F
Weight (kg)	172.67 ± 27.0	9.15	130 - 262	
BCS (/5) (n=30)	Median: 3	NA	3.0 – 4.5	
Age (years)	20.58 ± 11.9	4.02	3 - 45	
Heart rate (bpm)	48.80 ± 7.5	2.54	38 - 70	
				Differences between genders (P value)
Geldings Weight (kg)	Median: 173.5	NA	138 – 215	
Females Weight (kg)	Median: 163	NA	130 - 262	P = 0.072
Geldings BCS (/5)(n=21/24)	Median: 3	NA	3 – 4.5	
Females BCS (/5)(n=9/12)	Median: 3	NA	3 - 3.5	P=0.435
Geldings Age (years)	19.0 ± 12.5	5.26	3 – 45	
Females Age (years)	23.75 ± 10.3	6.57	6 – 36	P=0.264
Geldings heart rate (bpm)	50.04 ± 8.3	3.5	41 - 70	
Females heart rate (bpm)	46.5 ± 5.4	3.4	40 - 56	P=0.189

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308 Abbreviations: BCS; body condition score, bpm, beats per minute, NA not applicable.

309 **Table 2A: M-mode echocardiography results from 36 donkeys**

	Mean ± SD	Confidence interval (CI) of the mean	Minimum	Maximum
RVDd (mm)	20.7 ± 3.9	1.3	13.5	30.4
IVSd (mm)	16.2 ± 2.4	0.80	10.7	22.4
LVDd (mm)	68.1 ± 6.7	2.3	54.0	85.7
LVFWd (mm)	17.0 ± 2.6	0.9	11.1	23.2
IVSs (mm)	25.8 ± 3.5	1.2	18.8	33.4
LVDs (mm)	40.7 ± 5.2	1.8	29.3	51.2
LVFWs (mm)	22.7 ± 3.6	0.6	15.4	30.3
FS (%)	39.7 ± 7.3	1.2	27.0	56

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311 **Table 2B: M-mode echocardiography results: Males (N=24) and Females (N=12) separated**

	Mean ± SD (males)	CI of the mean (males)	Mean ± SD (females)	CI of the mean (females)	P value
RVDd (mm)	21.8 ± 4.0	1.7	18.5 ± 2.8	1.8	0.140
IVSd (mm)	16.4 ± 2.3	1.0	15.7 ± 2.6	1.6	0.458
LVDd (mm)	**66.5 ± 6.3**	2.7	**71.3 ± 6.6**	4.2	**0.022**
LVFWd (mm)	16.8 ± 2.6	1.1	17.4 ± 2.8	1.8	0.480
IVSs (mm)	25.5 ± 3.6	1.4	26.4 ± 3.9	2.5	0.461
LVDs (mm)	40.3 ± 5.0	2.1	41.5 ± 5.8	3.7	0.542
LVFWs (mm)	23.0 ± 3.6	1.3	22.2 ± 3.50	2.2	0.521
FS (%)	39.0 ± 7.1	3.0	41.0 ± 7.6	4.8	0.460

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Significant differences between genders are indicated in bold.

Abbreviations: SD: standard deviation, RVDd: Right ventricular diameter in Diastole, IVSd: Interventricular septum thickness in diastole, LVDd: left ventricular diameter in diastole, LVFWd: Left ventricular free-wall thickness in diastole, IVSs: Interventricular septum thickness in systole, LVDs: left ventricular diameter in systole, LVFWs: Left ventricular free-wall thickness in systole, FS: fractional shortening.

Table 3A: 2D echocardiography results from 36 donkeys

	Mean ± SD	Confidence interval (CI) of the mean	Minimum	Maximum
LA Long axis maximal diameter (systole) (mm)	63.7 ± 6.3	1.1	46.8	74.3
LA diameter d (short axis) (mm) (n=34)	54.0 ± 5.2	1.8	43.7	63.4
Ao diameter d (short axis) (mm) (n=34)	40.8 ± 4.3	1.5	32.9	48.7
LA/Ao ratio (n=34)	1.32 ± 0.1	0.05	1.08	1.65
Ao diameter valve s (long axis) (mm)	35.6 ± 3.9	1.3	29.5	43.2
Ao diameter s, sinus of Valsalva (mm)	42.0 ± 4.5	1.5	33.2	52.5
Ao diameter s, sinotubular junction (mm)	35.7 ± 3.8	1.3	28.4	43.6
PA diameter (mm)	32.2 ± 4.10	1.4	25.8	43.3
Ao/PA ratio	1.27 ± 0.16	0.05	0.89	1.5

Table 3B: 2D echocardiography results: Males (N=24) and Females (N=12)

	Mean ± SD (males)	CI of the mean (males)	Mean ± SD (females)	CI of the mean (females)	P value
LA Long axis maximal diameter (systole) (mm)	64.6 ± 5.5	2.3	61.8 ± 7.6	4.9	0.204
LA diameter d (short axis) (mm) (n=34)	**55.9 ± 4.4**	1.9	**50.6 ± 5.0**	3.2	**0.004**
Ao diameter d (short axis) (mm) (n=34)	41.0 ± 4.4	1.9	40.4 ± 4.5	2.8	0.684
LA/Ao ratio (n=34)	**1.36 ± 0.13**	0.06	**1.25 ± 0.09**	0.06	**0.015**
Ao diameter valve s (long axis) (mm)	35.5 ± 3.8	1.6	35.6 ± 4.2	2.7	0.947
Ao diameter s, sinus of Valsalva (mm)	42.0 ± 4.6	1.9	42.2 ± 4.4	2.8	0.874
Ao diameter s, sinotubular junction (mm)	35.42 ± 3.50	1.5	36.4 ± 4.3	2.7	0.475
PA diameter (mm)	32.36 ± 4.18	1.8	31.9 ± 4.1	2.6	0.749
Ao/PA ratio	1.27 ± 0.18	0.07	1.23 ± 0.18	0.07	0.450

Significant differences between genders are indicated in bold.

Abbreviations: SD: standard deviation, LA: left atrium, Ao: aorta, d: diastole, s: systole, PA: pulmonary artery (annulus), mm: millimetres.

Table 4: Association of Body Weight on Echocardiographic variables

	R	R^2	P	Linear regression equation for significant associations
M-mode variables				
RVDd			0.201	
IVSd			0.908	
LVDd			0.075	
LVFWd			0.051	
IVSs			0.139	
LVDs			0.161	
LVFWs	**0.343**	**0.118**	**0.041**	LVFWs = 14.94 + (0.045 x Weight Kg)
FS			0.680	
2D variables				
LA s max	**0.334**	**0.112**	**0.046**	LA LX s = 50.242 + (0.0780 x Weight Kg)
LA d	**0.474**	**0.225**	**0.005**	LAd d = 38.739 + (0.0886 x Weight Kg)
Ao d	**0.417**	**0.174**	**0.014**	Ao d = 29.566 + (0.0651 x Weight Kg)
Ao valve s	**0.579**	**0.35**	**<0.001**	Ao valve d = 21.324 + (0.0825 x Weight Kg)
Ao S of V s	**0.495**	**0.245**	**0.002**	Ao S of V d = 27.939 + (0.0816 x Weight Kg)
Ao sinotub s	**0.558**	**0.312**	**<0.001**	Ao ST junc d = 22.370 + (0.0774 x Weight Kg)
PA	**0.352**	**0.124**	**0.035**	PA = 22.974 + (0.0534 x Weight Kg)

Significant associations are indicated in bold, and relevant significant linear regression equations are only provided for significant associations.

Abbreviations: For M-mode abbreviations, see Table 1. LA: left atrium (maximal diameter, long axis 4 chamber view), s: systole, LA d: Left atrium (short axis) diastole, Ao d: Aortic diameter (short axis, level of aortic valves), diastole, Ao valve s: Long axis aortic annulus diameter at valve insertion level, systole, Ao S of V s: aortic diameter (long axis) in sinus of Valsalva, systole, Ao sinotub s: aortic diameter at junction of sinus of Valsalva and tubular aorta (long axis), systole. PA: Pulmonary artery annulus diameter (during systole).

Figure legends

Figure 1: Illustrations of 2D measurements made with electronic callipers on echocardiographic images.

A. 2D right parasternal short axis view at level of the aortic valves, with optimization of the left atrial size. The aortic valves can be seen, closed early in diastole. The aortic diameter measurement is made following the line of the non-coronary and left coronary cusps, and left atrial measurement is also shown, following this line.
B. 2D right parasternal long axis view, with optimization of the left atrial diameter. Maximal left atrial diameter is measured at the end of ventricular systole, before the mitral valve leaflets open.
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Figure 3D: 2D pulmonic annulus diameter (systole) and body weight

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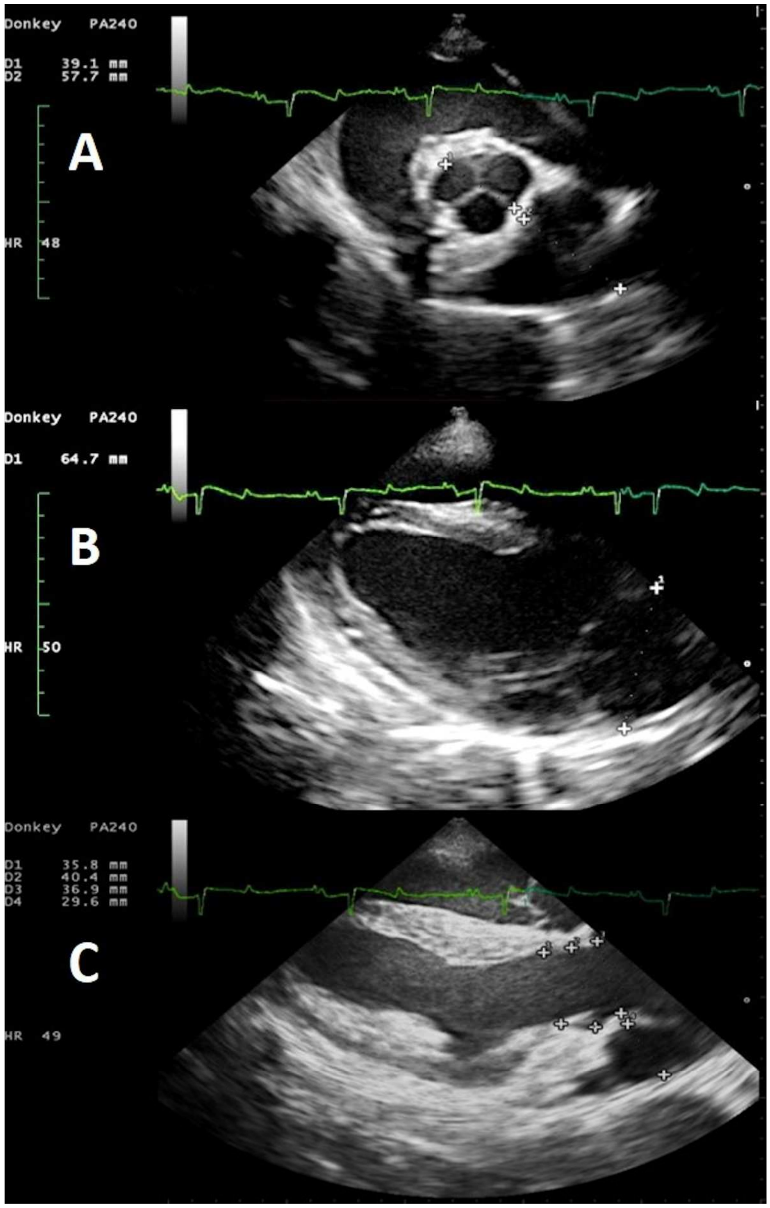

Figure 1: Illustrations of 2D measurements made with electronic callipers on echocardiographic images.
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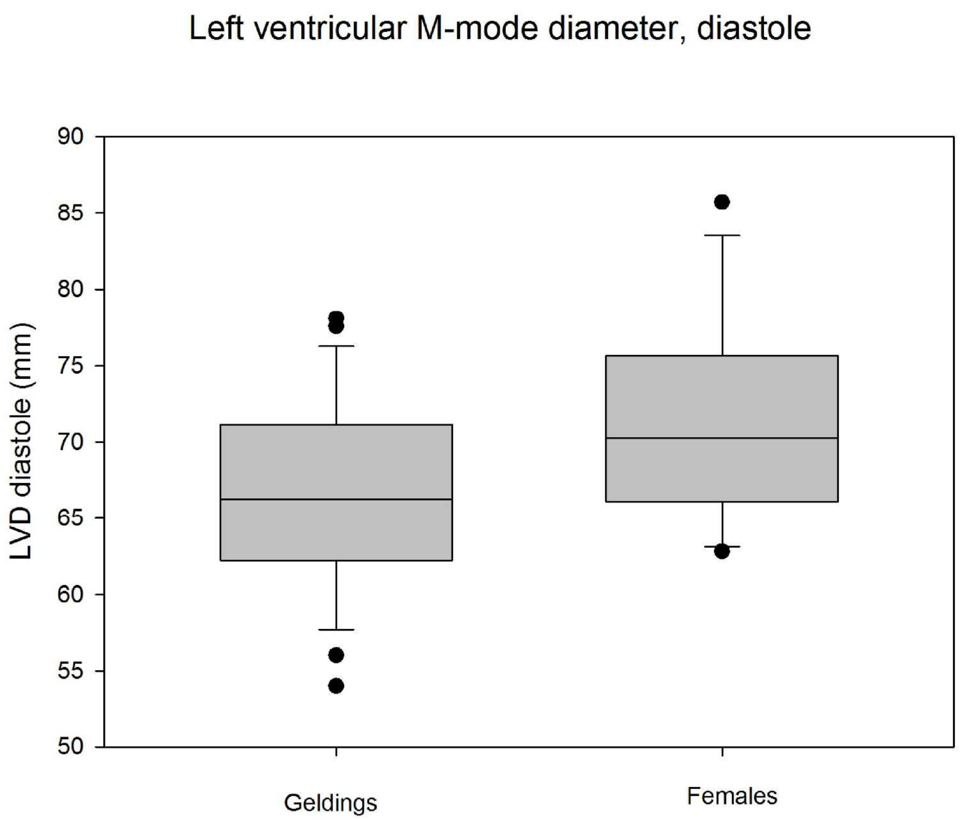

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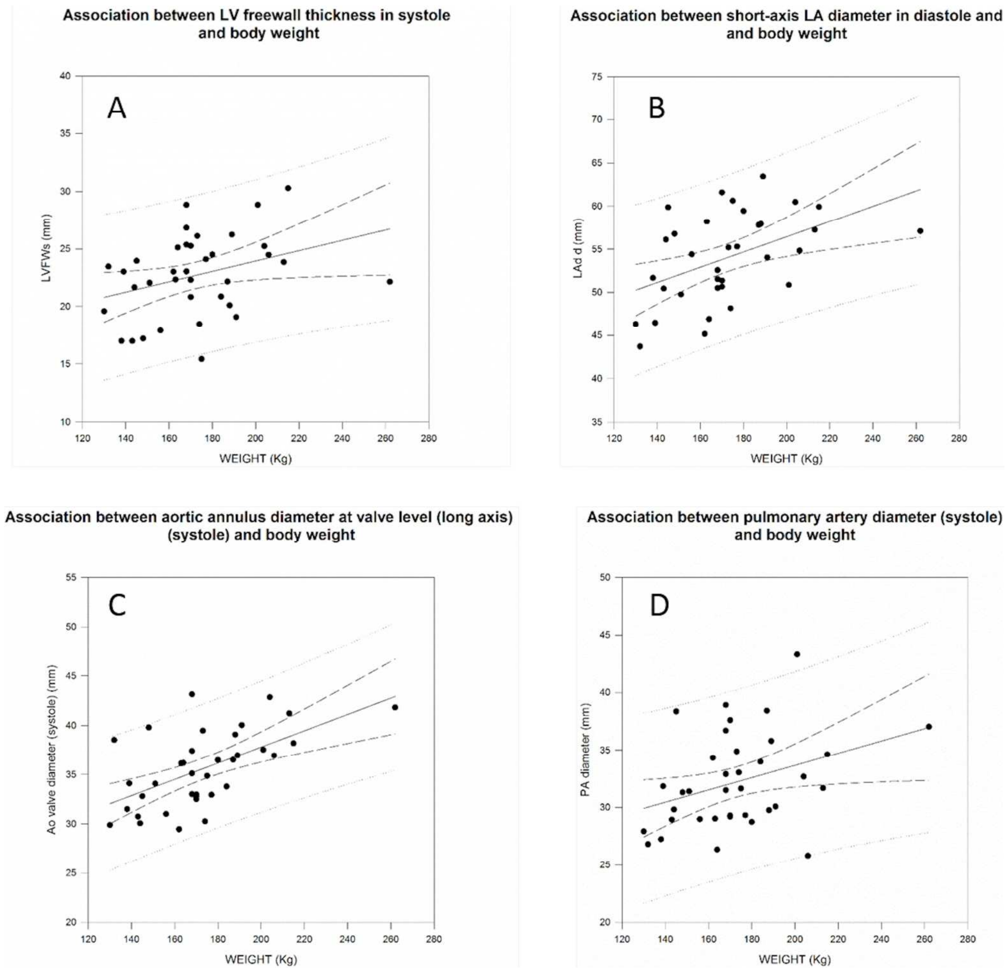

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Possible figure for front cover if desired by the editor.

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